Mahendralal Sircar Memorial Lecture, 1969

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Calcutta-32

(*Delivered on 22 September*, 1969)

Fellow Scientists, Ladies and Gentlemen,

I am deeply sensible of the honour of being invited to deliver Mahendralal Sircar Memorial Lecture for 1969. A pioneer of rare quality, Dr. Mahendralal Sircar was endowed with unequalled imaginative richness and scientific perception. The Indian Association for the Cultivation of Science is his monumental work, an imperishable legacy of a great endeavour.

To-day we really celebrate the centenary of Dr. Sircar's endeavour to establish the originality and identity of Indian science. Writing in 1869 in the Calcutta Journal of Medicine he gave us the first steps we should take in formulating any national plan for the development of science and technology in the country. Dr. Mahendralal Sircar was a medical man and medicine was then wrapped in an atmosphere of empiricism. It is really amazing that from wayside he could watch the triumphant march of the quickening spirit of physical sciences and rapid advances which brought wealth and prosperity to Europe as a whole. A magnificent man as he was, his vision transcended the barriers of confused thinking. It is to the undying credit of Dr. Sircar that he managed to instil a perception of science at a time when thinking was clouded by medievalism.

In order that a proper climate could be had for pursuit of research, Dr. Sircar thought boldly of an institution dedicated to science for the Indians, of the Indians and by the Indians.

Science is international but scientists are national. This was true as much for Mahendralal Sircar as for Louis Pasteur. And in adhering to this truth unflinchingly he faced many difficulties and privations but the challenge was accepted and the response, as you see it to-day, was a national laboratory of international fame, the product of his singular effort and neverceasing pursuit of uncompromising idealism.

It does give me much pain to note that even after independence the Indian Association for the Cultivation of Science remained a Cinderella while the Government could be Prince Charming to so many institutions.

This institution carrying as it did a rich legacy deserved very special attention. Yet it lacks equipments of modern specialized variety even to-day. Much remains to be done in this regard. This is hardly a fitting

tribute to the pioneer of pure science in India who laid such enduring foundations for scientific research.

When I took the decision of locating the Indian Institute for Biochemistry & Experimental Medicine at the adjacent building which was then a marshy low land with tanks and gulleys, I had faced criticism but was not deterred as I had felt somewhat thrilled by sheer historicity of the site. I was clear in my mind that I was taking the Institute into a scientific colony amidst which stood with passionless grandeur the epitome of pure scientific endeavour : the Indian Association for the Cultivation of Science. To be anywhere near it is to breathe in an atmosphere of research.

Such has been the lure of heritage of the Indian Association for the Cultivation of Science, yet the institution itself suffered gross neglect. The Government grants are woefully inadequate. So are the equipment and personnel as a result. The remedy lies in the Central Government stepping in and helping the Institute to expand on modern lines.

With the above observations, I would like to pass on to the theme of my lecture *viz.* the tools and techniques of microbiological research.

Tools and Techniques of Microbiological Research

In all branches of biological research to-day, new techniques and instrumentation have opened out vast areas for investigation and provided the means for the rapid advancement of knowledge. This has come about mainly as a result of development in applied physics—optics, electronics and electrical technology—and the intensive application of physico-chemical concepts and techniques in biological research. We have in fact reached a stage when it is necessary sometimes to guard against the tendency to exaggerate the importance of instrumentation and techniques and to consider them an end in themselves. However powerful and versatile the tools available to the research worker, he can get little out of them unless he uses them purposefully and for fruitfully conceived ends—the scientific objective of his study. In this sense, the human brain retains, as it will always retain, its unchallenged pre-eminence as the means to the acquisition and extension of scientific knowledge.

Nevertheless, it is important for the working scientist to keep abreast of advances in techniques and instrumentation in his field, for they are the tools of his trade and the quality of his work will be determined by his expertise in their use. The development of science is conditioned not only by the social milieu in which the scientist works, but also by the tools and techniques available to him for his work. Thus, the classical investigations on X-rays and elementary particles followed from the

development of techniques for producing high vacua, and the discovery of the rare gases from accurate techniques of quantitative chemical analysis. In this sense techniques and instrumentation set not only the pace of progress in science but also the directions of that progress.

The role of instrumentation and techniques in the advancement of science is most aptly illustrated in the history of microbiology. Microbiology as a science was born with the invention of the microscope. The use of the microscope, in combination probably with the method of dark ground illumination enabled Leeuwenhoek to discover bacteria and describe practically all the morphological types of them known today. The technique of cultivation of bacteria in pure culture which Koch established opened up inexhaustible fields of research and influenced the whole subsequent history of bacteriology ; this, in spite of the fact that the method was simplicity itself. In Koch's own words "the peculiarity of my method is that it supplies a firm, and where possible, a transparent pabulum ; that its composition can be varied to any extent suited to the organism under observation ; that all precautions against the possibility of after-contamination are rendered superfluous ; that subsequent cultivation can be carried out by a larger number of single cultures of which, of course, only those cultures which remain pure are employed for further cultivation ; and that, finally, a constant control over the state of the culture can be obtained by the use of microscope".

Next only in order of importance to the development of the science of bacteriology was the establishment of techniques for staining bacteria. The employment of stains for bacteria followed from their earlier use in ordinary histological work. This started with carmine preparations which were originally employed by Goeppert and Cohn but came into prominence through the work of Hartig and later, Gerlach. The preparation of dyes from aniline and coal tar took place about this time and greatly influenced staining techniques in bacteriology. Weigert showed that methyl violet can be successfully used to reveal cocci in tissues, and other workers stained bacteria in an aqueous solution of fuchsin. But again, it was Koch who laid the foundation of the technical procedures employed today for staining. Realizing the importance of getting the bacteria into a non-motile state, he prepared thin films on cover glasses and dried them. He then fixed the preparations with alcohol and applied various stains, and finally mounted the preparations in Canada balsam. From then onwards staining methods were rapidly perfected, much of it on the basis of the epoch-making work of Ehrlich on the staining of specific granulations in white blood corpuscles.

Proceeding from these three basic techniques—microscopy, pure cultivation and staining—I shall rapidly review recent developments which have augmented and diversified their scope and application in bacteriological research and consider their impact on current objectives of microbiological study. For the examination of living cells, phase contrast microscopy has become the method of choice. This method, first described by Zernike in 1942 has been in general use for over fifteen years and I need not describe it at length. In conventional microscopy a transparent object remains invisible, because it has no effect on the intensity of light while the eye or the photographic plate are only sensitive to variations in light intensity. Nevertheless, a transparent objective influences light passing through it by delaying or altering the phase or optical path to an extent determined by its thickness and refractive index. In a heterogeneous transparent object such as a living cell there are regions of different thickness and refractive index. Each such region will therefore introduce a phase change in a light wave passing through it. These invisible phase changes are converted into intensity changes and a transparent object made to appear as if it were an absorbing object in the phase contrast microscope by separating the original and the diffracted wave and altering thier relative phase contrast before recombining them. Briefly, the practical achievement of phase contrast depends on the ability to separate the original and diffracted waves.

It will be evident that the development of the phase contrast microscope represents a dual advance in technique ; it not only extends the scope of microscopic observation but provides at the same time what may be described as an optical equivalent to chemical staining techniques. Staining procedures designed to reveal details of sub-cellular structure reduce the dynamic morphological features of the living cell to a static condition, and destroy or distort the vital activity of the features under observation. The phase contrast microscope enables structures to be observed without chemical interference with normal cellular activity.

However the phase contrast microscope suffers from certain shortcomings, chiefly the appearance of a halo around every object-detail and the non-uniformity of the phase contrast effect which tends to fall off towards the centre of large uniform objects. The halo effect is reduced or eliminated in some forms of interference microscopes, which are thus more suitable for quantitative work. The basic theory underlying the operation of the interference microscope, is that a transparent object can be made to appear like an absorbing one by adding a suitable wave, instead of as in phase contrast, by altering the phase relationship between the direct and diffracted waves.

An important application of both the phase contrast and interference microscopes is in the measurement of the concentration of solids and the mass of cells. In both types of microscopy image contrast depends upon the optical path difference or phase change induced by the object, which in turn is a function of its refractive index. It is thus possible to determine the refractive index of the object under observation, from known mathematical relationships. The technique of cell refractometry consists essentially in varying the refractive index of the immersing medium until those parts of the cell in contact with the medium disappear. Since many careful measurements have shown that the relationship between refractive index and the concentration of dissolved substances is linear, it becomes a simple matter to calculate the concentration of solids (and dry mass of the cell) from the observed value for refractive index. The dry mass may also be calculated from direct measurement of the phase change using the interference microscope. However, there is no simple method at present of determining the total dry mass of an entire cell, although it is comparatively easy to determine the dry mass per unit area of any region of a cell.

Cell refractometry, applied to large populations of cells, has already yielded valuable information about the solid concentration in many species of bacteria grown on solid and liquid media. Refractometry has also been used for the study of fungal growth. Mitchinson used interference microscopy to show that the increase in the dry mass of a single yeast cell was approximately linear upto the stage of division, whereas the solid concentration was fluctuating.

Another important advance in the employment of special types of microscope has been the development of the ultraviolet microscope. It gives photographic records indicating difference in absorption of short wave ultraviolet light by particular substances, especially nucleic acids. Fluorescence microscopy has found even wider application ; although the first fluorescence microscope was constructed by Kohler more then fifty years ago, it is only during the last ten years that the instrument has become prominent in research, notably in the fields of immunology and virology. In it the specimen itself is rendered luminous and emits light to form the observed image. The high pressure mercury vapour lamp is now used in most laboratories as the most convenient source for excitation of the specimen and this is combined with a collecting lens, and an appropriate long wave ultraviolet or blue glass filter.

The prevailing interest in fluorescence microscopy is due largely to the fluorescent antibody technique, which originated with the discovery of Coons & Kaplan in 1950 that antibody globulins from the blood of

immunized animals could be linked to a fluorescent compound, fluorescein isocyanate, without loss of their capacity to combine with their specific antigens. Owing to the characteristic pale green fluorescence of the fluorescein-treated antibody, it was possible, with a fluorescence microscope, to detect for the first time in tissues the exact sites of interaction between the antibody and the antigen. The technique may be modified for locating the site of antibody production in immunized animals and for detecting antibody protein deposited in abnormal situations.

One of the most rewarding applications of the fluorescent antibody technique has been in the study of various phases of virus growth in cell cultures or in sections of infected tissue. Some components of viral protein at an early stage of synthesis, as well as aggregations of the fully formed virus particles can thus be located. In certain instances different viral components are seen to be synthesized in separate parts of the host cell and to be combined later in the assembly of the mature virus.

Fluorescent antibody reagents are also finding increasing application in the study of auto-immune diseases, in which antibodies are formed against certain elements of the patient's own tissues. Thus in Hashimoto's disease, an antigen-antibody reaction has been observed in the thyroid colloid and thyroid cells of the patient from whom the fluorescein-antibody complex had been prepared. More recently, a positive reaction of this kind has been observed in heart muscle with fluorescent antibody prepared from the serum of patients with rheumatic heart disease.

The development of fluorescence microscopy has in turn led to important advances in staining technology. Highly fluorescent substances, the fluorochromes are being used as direct stains for fixed tissue preparations. The use of the fluorescent stain auramine for the quick identification of tubercle bacilli in sputum and other specimen is now common practice. The compound acridine orange is a fluorochrome of special interest, as under controlled conditions it is selectively bound to cell components containing nucleic acids. This method has proved particularly useful in virus research owing to its sensitivity and the invariable presence of nucleic acid, either DNA or RNA as an essential part of all viruses. The normal nucleic acid pattern of the host cell is profoundly altered in virus infections and significant changes of this kind have been demonstrated microscopically, using acridine orange, during the growth of influenza virus, adenovirus, polyoma virus and others.

The special types of microscopes so far considered share the same fundamental basis, the use of light to illuminate the specimen and of a series of lenses operating on and magnifying the object itself as well as

the images produced of it. They share a common limitation also, for the detail that can be observed in the image produced by them is limited to structures larger then about one-fourth of a micron. This limit is the wave-length of light and a permanent restriction to the resolution of such detail. Some thirty five years ago research on the properties of electron beams led to the idea of a microscope in which electron beams and magnetic fields replaced visible light and glass lenses. The extremely short wave-length of an electron beam suggested that an image might ultimately be obtained with such an instrument showing detail even below one-thousandth of a micron (or one-millonth of a millimetre) which is roughly the size of the diameter of the polypeptide chain in a protein molecule. The electron image itself is not visible but like X-rays, electrons can excite a fluorescent screen or darken a photographic film so that the image may be viewed and recorded.

The first attempt to build an electron microscope was made by Knoll & Ruska in 1932. This eventually led to the production of the first commercial electron microscope by von Borries & Ruska about 1938. The instrument utilized magnetic lenses entirely and its resolution approached 2 millimicrons. In the last 25 years the performance of the electron microscope as an instrument has been improved by a factor of only 2 or 3, but the limit in the resolution of objects now reached is not far short of the size of the atom. The detail seen in practice is however much more seriously limited by the conditions under which the enlarged electron image has to be obtained. Advances in technique have now overcome many of the limitations set by these requirments, but the full theoretical potentialities of the instrument have not even yet been completely realized. Electron microscopes are being applied to biological problems at resolutions representing again of over 100 times beyond the light microscope. A futher increase by a factor of 10 is theoretically possible.

The essential requirement for electron microscope specimens is extreme thinness, and for obtaining sufficient contrast in the picture, density approaching that of a metal. The difficulties of maintaining the architecture of biological material so thinly sliced have been largely solved. A satisfactory cutting edge was found in broken pieces of ordinary plate glass. A widely used method for increasing the contrast of direct specimens is that of metal shadow casting. More recently a technique has been developed for the deposition of viruses on support films in which virus particles are deposited from an extremely strong solution of phosphotungstic acid. After evaporation of the liquid vehicle and examination in the electron microscope, the phosphotungstic acid is seen to surround

the virus particles as a dense background matrix which allows the virus to be observed as a negative image. By this technique many surface details of the virus are preserved, which are lost if the virus is examined directly.

The impact of the electron microscope on microbiological research, especially the stduy of viruses, can only be described as revolutionary. With few exceptions, viruses are of a size well below that of objects visible under a light microscope, but are ideal objects for study by the electron microscope as they do not require to be sectioned. The electron microscope has in fact already yielded exact and detailed knowledge of the structure of viruses, and shown up almost every surface feature of many of them. It provides the only exact methed of estimating the number of virus particles in a suspension and of measuring the rate at which viruses arrive at a surface such as that of a cell, which they are going to infect. This rate of arrival at a surface has been shown to depend on random Brownian movement.

The electron microscope proves potentially useful in the classification and identification of bacteria. Previously and even now morphology and chemical activities are being used only as criteria for the purpose. Electron microscope has revealed much greater details in the structure and morphology of bacterial cells. Recently attempts are therefore being made for classification and identification of bacteria purely on electron-micrographs. Host-virus interrelationships form the basis of viral classification and identification. Now electron-microscope has revealed much finer details in viral surface structures. Hence work is now in progress to classify and identify viruses purely on the basis of morphological details. Electron microscopy coupled with staining has demonstrated the existence of cell wall and protoplasamic membrane in bacterial cells. That the membrane is constituted of three distinct layers is another advance in the study of the structure of bacterial cells.

Electron microscope has proved to be of invaluable help in the study of sub-cellular particles not only of bacterial but also of mammalian cells. Electron-micrographs of sub-cellular particles like mitochondria, microsome, nucleus, chromosome etc., have revealed much valuable informations not only as to their structures and dimensions but also to their function in the biology of the cell. Recently electron microscopy has revealed the existence of another class of sub-cellular particles called polyribosome which is essentially a polymer of ribosome. Its discovery has brought us one step forward in our understanding of the genetic transcription and translation process and hence protein synthesis.

Genetic exchange by way of mating has been demonstrated first by Lederberg in E. Coli K12 with the help of auxotrophic mutants. Reversion to prototrophy over the spontaneous mutation rate has been taken as one of the criteria of genetic exchange or sexuality. This is further confirmed by electron microscopic observations which has revealed the presence of a clearly visible transparent bridge between conjugating pairs in a bacterial population.

Clearly, the results so far obtained mark only the beginning of the contributions of electron microscopy to microbiological research. An instrument that places in our hands the means to see, and count, and measure objects such as viruses, which formerly could be detected only by indirect methods, has almost limitless potency as an aid and indeed a stimulus to research.

Among advances that bear on the technique of cultivation in pure culture, recent developments relating to the continuous culture of micro-organisms merit special notice. The evolution of devices for maintaining cell suspensions in continuous growth originated in efforts to provide growing micro-organisms with frequent replenishments of nutrients, preferably with an apparatus into which the food supply could be introduced mechanically. Two classes of instrument allow automatic addition of nutrient and the attainment of a steady state at a relatively unifrom population density of growth rate. The first class add nutrient in response to increased turbidity of the culture, thus maintaining a uniform density through dilution and wash-out of cells. In the second category, exemplified by the chemostat of Novick and Szilard, and the bactogen of Monod, growth is kept at a constant level by a growth limiting factor supplied at a fixed rate of input. A modification of the chemostat also enables its use as an instrument working by turbidity control.

The maintenance of a uniform level of turbidity of the culture is achieved through control devices generally known as turbidostats, operated by a system of photocells. Disperson is provided by a magnetic stirrer, and various adjustments control the time of operation of the pump delivering nutrient to the culture volume on actuation by the photo-electric device, volume of fluid delivered per stroke of the pump, heating rate of the culture, rotational velocity of the stirrer and other parameters. Sterile air is continuously provided under pressure to the air space above the culture, escaping to the atomsphere by exit at the top of the growth tube. The turbidostat has been mainly used for growing bacteria.

In the chemostat, the bacterial suspension contained in a growth tube is fed fresh nutrient at a rate set by the experimenter. The contents

of the tube are mixed and aerated by bubbling air. The bacterial suspension is removed from the tube at the same rate at which fresh nutrient enters it, and for this reason the volume of suspension in the growth tube remains constant. The rate at which the contents of the tube are diluted by the flow of liquid through it is kept less than the maximum rate of growth exhibited by the bacteria in the entering medium.

Mass propagation of micro-organisms is the most direct application of continuous culture methods and naturally it is in fermentation technology that prominent use has been made of the method. In the manufacture of yeast as well as alcohol by yeast fermentation, continuous culture techniques have been found to be specially advantageous. They have also found application in the large scale production of algae as a potential source of food, and as a source of oxygen for the continuous treatment of sewage.

Pilot plant experiments are under way for fermentative production of enzymes. Work on steroid, transformation synthesis of dextran and antibiotics like penicillin, streptomycin, chloro-tetracycline, novobiocin, chloramphenicol, studies on production of B_{12} from wastes, proteins from hydrocarbons, acetone-butanol, citric acid, glycogen etc., are however, confined as yet to academic research.

However, continuous culture methods are readily applicable on the laboratory scale also, and ideally suited for the study of important aspects of the biology of micro-organisms, such as the influence of environmental conditions on growth and biosynthetic activities of growing cells. For yield measurements, production of antigenic components and studies of population genetics and interaction of mixed bacterial populations, continuous cultivation is obviously the method of choice, and has already yielded results of fundamental interest.

Besides the instrumentation techniques as referred to above, recent years have seen tremendous progress in microbial genetics and biochemical genetics in particular due to the development of some biological techniques like mutation, conjugation, transformation, transduction, radiobiological techniques etc.

After the discovery of mutation in Drosophila, by Muller, the method has been successfully applied by Beadle in an ingenious way to Neurospora in which gene changes have been in the ultimate analysis equated to biochemical alterations. This leads to the postulation of one gene one enzyme theory. Recent researches of Jacob and Monod further modified one gene one enzyme theory and established multigenetic control of protein synthesis. Mutation technique has another

useful application in the development of potent strains in fermentation technology. As a matter of fact the tremendous reduction in the production cost of penicillin is primarily because of two reasons, firstly development of potent strains and secondly the quick separation of penicillin by centrifugation technique. Auxotrophic mutants as isolated by classical method of Beadle have been in use for the bioassay of vitamins and aminoacids. Mutation production in TMV coupled with amino-acid analysis of coat proteins by finger print method has also greatly helped in establishing the genetic code.

In recent years mutation technique itself has been much simplified. The isolation of mutants by total isolation method of Beadle is an extremely painstaking and laborious task. Penicillin enrichment technique discovered independently by Lederberg and Davis, coupled with Replica plating has tremendously simplified the isolation procedure. The method is however applicable only to penicillin sensitive gram positive organisms. Penicillin may be replaced by azaguanine in case of penicillinase producing or penicillin insensitive organisms. Filamentous fung may be tackled by filtration technique for the isolation of auxotrophic mutants.

Transformation, another important biological technique, has identified "genes" of biologists with "DNA" of chemists. It will pave the way for our understanding of the sexual mating process on a molecular plane. Genetic homology may be a convenient method of testing sexual compatibility. Transduction, another method of genetic exchange, also discovered by Lederberg *et al*, has proved extremely useful in the analysis of fine genetic structure.

The cultivation of mammalian cell culture is another method that has been steadily gaining in prominence in microbiological research. The technique of tissue culture had been standardized at far back as 1923, and from the beginning its incalculable value in virological research was fully realized. Cell culture methods have now been developed for quantitative assay of viruses, for diagnosis and for the production of vaccines. However, the potentialities of tissue culture in other areas of microbiological study have not been as yet fully exploited. It is only recently that tissue culture has begun to be used in the study of intracellular organisms like the Rickettsiae, the myco-bacteria and the pleuropneumonia organisms. The results so far obtained clearly indicate how fruitful the technique can be in such work. Lepraemurium has been successfully grown, for example, in the strain L cell and detailed information obtained about the growth, life-cycle and metabolism of Rickettsiae. These organisms are all either slow-growing or else dependent on the

presence of living cells and tissue culture is the logical method for their investigation. But of equal promise is work, that is only beginning, on more rapidly growing pathogenic organisms using the fast-growing cells strains that have been recently introduced. These investigations should advance knowledge of host-parasite relationships at the cellular level, especially the biochemical bases of pathogenicity which are apparent only in organisms grown *in vivo* or in the cellular environment necessary for the full induction of their pathogenic capacity.

Cell culture technique has neen of great use in the study of mammalian biochemistry on a cellular level. Its importance in immunological research can hardly be described. Some of the important questions of immunology *e.g.*, active site of antigens, biogenesis of antibodies *etc.*, which have been a puzzling block for a pretty long time may now be suceessfully tackled on a cellular level. The biochemistry of many genetic and metabolic diseases can be more conveniently studied with the help of cell-culture technique. The technique may be used for fermentative production of many animal producte, *viz.*, insulin, antibodies etc. Organ culture, although not a microbioiogical but definitely a mammalian cell culture technique, has immense future possibilities.

Amongst important advances in techniques of characterization I have already referred to fluorescence microscopy and the fluorochrome stains. Mention should be made of two other methods which have recently become available, cell electrophoresis and the partition of cells in aqueous polymer two-phase systems, which are applicable to the characterization as well as isolation of micro-organisms. Both these techniques originated from work on the fractionation of proteins and other macro-molecules at the Institute of Biochemistry in Uppsala. The theoretical basis of cell electrophoresis is the uniform and characteristic electrical charge of cells in a population deriving from the same strain and grown under identical cultural conditions. The cells therefore possess a uniform and characteristic electrophoretic mobility. The method involves direct microscopic observation of the cells as they migrate in the electric field. The cells are suspended in a suitable buffer solution and placed in a small flat or cylindrical transparent cell through which an electric current is passed. The time for a cell to cover a given distance as measured with a micrometer eyepiece is determined and from this the mobility or velocity per unit field strength may be calculated. For preparative purposes a different design is necessary, but a fully satisfactory arrangement has still to be evolved. Cell electrophoresis has been applied in the measurement of the mobilities of viruses absorbed on collodion particles, and the effect thereon of strain differences and

chemical treatment. It has been shown that the mobility of a given strain of bacteria is characteristic of that strain and remains constant through many sub-cultures. The method has also been found useful for distinguishing between sub-groups within a species, especially variants of the rough-smooth type and for studying the action of detergents and antibiotics on bacterial surfaces.

The partition of cells and cell paricles by distribution in liquid-liquid two-phase systems is also based on the specificity of their surface properties. Separation of substances by partition is a classical and perhaps the most widely used method in organic chemistry. In biochemistry most of the partition methods were originally developed for low-molecular substances like peptides, hormones and vitamins and subsequently modified, as in partition chromatography, for the separation of proteins, enzymes and nucleic acids. Special problems which arise when a phase system has to be applied to particles of biological origin have been solved by Albertsson through the use of an aqueous polymer two-phase system yielding phases rich in water and allowing a reproducible partition of even fragile particles and macromolecules under mild conditions. The method has not been fully explored in microbiological research, but offers important possibilities, in the concentration and purification of viruses and antigen-antibody complexes, and the differentiation of bacterial strains.

Early fifties have seen tremendous advance in the study of the chemistry of bacterial cell walls, when the mode of action of penicillin has been fairly worked out. In the preparation of pure cell walls free from cytoplasmic contaminants low speed differential centrifugation has been of great use. The preparation of pure cytoplasmic membranes may be conveniently achieved with the help of differential centrifugation technique.

To this must be added ultracentrifugation the importance of which in separation technique needs no exaggeration. The separation of sub-cellular particles like mitochondria, microsome, chromosome etc., depends entirely upon the use of ultracentrifugation. Further fractionation of sub-cellular particles, *e.g.*, microsomes into ribosomes, its constituent 70S, 30S or 50S particles *etc.*, depends upon successful application of differential ultracentrifugation. The usefulness of the separation of sub-cellular particles in developing our present concept of protein synthesis and in establishing the universal genetic code and also its degeneration, can hardly be described.

The application of biochemical methods in the study of microbial metabolism, particularly paper chromatography, and radio-isotope techni- has extended the horizon of knowledge in sciences, and provides a spectacular example of the cross-fertilization of ideas in sciences. In fact biochemical techniques have been assimilated as an essential and integral part of microbiological methodology.

I have attempted in this review to give only a bird's eye view of the present state of micro-biological methodology. I have perforce had to take a selective view, largely based on my own particular scientific interests, in choosing the development I have discussed as typical of the historic growth of microbiology and exemplifying the present status of its techniques and instrumentation. But I believe there can be no disagreement over the conclusion I would put before you, that the techniques we have now available have opened out vast new areas for investigation and provide a most potent stimulus to research in all the separate and specialist disciplines of study which the science of micro-biology now comprehends.